

S/N 10/542,914

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Alfred Marchal	Examiner:	Valenrod, Yevgeny
Serial No.:	10/542,914	Group Art Unit:	1621
Filed:	March 7, 2006	Docket No.:	09997.0127USWO
Customer No.:	23552	Confirmation No.:	9556
Title:	USE OF A COMPOSITION COMPRISING VITAMIN K1 OXIDE OR A DERIVATIVE THEREOF FOR THE TREATMENT AND/OR THE PREVENTION OF MAMMAL DERMATOLOGICAL LESIONS		

DECLARATION OF ALFRED MARCHAL UNDER 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

Dear Sir:

1. I, Dr. Alfred Marchal, declare as follows:
2. I am an inventor of U.S. patent application no. 10/542,914 (hereinafter the '914 application) filed July 20, 2005. The '914 application is a national stage application of international application no. PCT/BE/04/0011 filed January 20, 2004.
3. I have a Ph.D. from Brussels University in pharmacology and organic chemistry. I have conducted research in the areas of synthetic organic chemistry, organic fluorine chemistry, phytopharmacy, cardiovascular molecules, anti-malaria drugs, and racemic resolution techniques. I have extensive research experience in synthetic organic chemistry with a focus on development of original pharmaceutical molecules. I am currently the Chief Executive Officer of Auriga International, a pharmaceutical company specializing in research and development of dermatology products.



4. Between 1964 and 1994, at least 52 patients with cutaneous adverse effects of vitamin K1 were described in the European and North American literature, with 94 cases in the Japanese literature. See Exhibit 1 at section 3.3.11, "Type IV hypersensitivity to vitamin K" and Exhibit 2 at page 77, section entitled "Réactions locales". The cutaneous adverse reactions were found to split into two groups: reactions of eczema type and reactions of scleroderma type. See Exhibit 2 at page 77, section entitled "Réactions locales".
5. Between December 2003 and June 2004, five cutaneous adverse side effects of allergic nature resulting from the use of three cosmetic products containing vitamin K1 were reported to the French drug security administration Agence française de sécurité sanitaire des produits de santé (hereinafter "Afssaps"). In response, Commercialization of these products was stopped by Afssaps on November 12, 2004. See Exhibit 3 at page 1.¹ On March 8, 2006, Afssaps prohibited the manufacturing, commercialization, sale, and use of cosmetic products containing vitamin K1 in France. See Exhibit 3 at page 1. In response to the reports of cutaneous adverse effects of vitamin K1, the European Commission Scientific Committee on Consumer Products concluded that the use of vitamin K1 in cosmetic products is not safe. See Exhibit 1 at section 4.
6. The cutaneous adverse side effects were not limited to vitamin K1. Therapeutic application of the synthetic analogue vitamin K3 had previously been investigated and abandoned as application of vitamin K3 1% in an ointment base to the skin produced an irritant contact dermatitis. See Exhibit 5 at page 96, first column.
7. In view of the knowledge within the field in at the time of filing of the PCT application (January 20, 2004) regarding cutaneous adverse side effects associated with the administration of vitamin K1 both topically and parenterally, I would not have considered vitamin K1 or a vitamin K1 analogue suitable for use in a

¹ Exhibit 3 is an English translation of the decision from Afssaps dated March 8, 2006 (Exhibit 4).

composition, such as a crème, gel lotion, or liquid, for treating a dermatological lesion.

8. As discussed above, at the time of filing of the PCT application (January 20, 2004) it was known that the use of vitamin K1 in cosmetic products was not safe as it may cause cutaneous allergy. Vitamin K1 was also known to be unstable in a cosmetic product when exposed to UV light. See the '914 application, for example, at paragraph [0003]. I therefore conducted a study in 2003 to determine the phototoxic potential of vitamin K1 and vitamin K1 oxide with respect to epidermis (Exhibit 6).
9. A phototoxicity assay was carried out using SKINETHIC™ human reconstituted epidermis (REPs). A fully differentiated epithelium having the features of human epidermis was obtained by culturing human keratinocytes in a chemically-defined medium on inert microporous polycarbonate filters at the air-liquid interface. See Exhibit 1 at page 4, first paragraph. Vitamin K1 and vitamin K1 oxide were tested undiluted (i.e. 100%) and diluted to 10% and 1% (v/v) in paraffin oil. Following an initial acclimatization at 37°C/5% CO₂ in air, samples of REPs were treated overnight (approximately 18 hours) in duplicate with 10 microliters of vitamin K1 or vitamin K1 oxide directly applied to the culture surface on the dry stratum corneum of epidermis. See Exhibit 6 at page 4, section 3.1. The toxic effect of vitamin K and vitamin K1 oxide was assessed using the MTT assay. Results under non-UV conditions are summarized in Table 1.

Table 1

Vitamin K1	
Concentration [% (v/v)]	Viability (%)
0	100
1	96
10	93
100	93
Vitamin K1 oxide	
Concentration	Viability

[% (v/v)]	(%)
0	100
1	90
10	91
100	100

As shown in Table 1, no significant cytotoxicity was observed in REps exposed to vitamin K1 or vitamin K1 oxide at the tested concentrations.

10. Undiluted (100% v/v) vitamin K1 or vitamin K1 oxide was applied to REps and incubated 24 hours prior to UV_A irradiation. The results under UV and non-UV conditions are summarized in Table 2.

Table2

Vitamin K1			
Viability (%)	Cytotoxicity (%)	Concentration [% (v/v)]	UV _A irradiation
100	0	0	
97	3	0	6 J/cm ²
100	0	100	
38	62	100	6 J/cm ²
Vitamin K1 oxide			
Viability (%)	Cytotoxicity (%)	Concentration [% (v/v)]	UV _A irradiation
100	0	0	
97	3	0	6 J/cm ²
100	0	100	
99	1	100	6 J/cm ²

11. As shown in Table 2, the viability of REps treated with vitamin K1 in UV_A irradiation conditions as compared to non-UV conditions was markedly decreased. These results indicated that vitamin K1 is phototoxic. In contrast, the viability of REps treated with vitamin K1 oxide in UV_A irradiation conditions was comparable to that in non-UV conditions.

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12. If vitamin K1 oxide were an analogue of vitamin K, it would be expected that vitamin K1 oxide would have activity in the phototoxicity assay similar to vitamin K1. The results in Table 2 show that vitamin K1 oxide is not phototoxic. These results were unexpected in view of the vitamin K1 results, which show that vitamin K1 is phototoxic.

13. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further, that these statements are made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful and false statements may jeopardize the validity of the application or any patent issuing thereon.



Alfred Marchal, Ph.D.

Dated: 2010/01/16 th

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Exhibits

- Exhibit 1: "Opinion on Vitamin K1 (Phytonadione)", *Scientific Committee on Consumer Products (SSCP)*, 24 June 2008
- Exhibit 2: Progrès en Dermato-Allergologie, Grenoble 2005
- Exhibit 3: Translation of the decision from AFSSAPS dated March 8, 2006
- Exhibit 4: Decision from AFSSAPS dated March 8, 2006
- Exhibit 5: Sommer et al., "Type of IV hypersensitivity to vitamin K", *Contact Dermatitis*, 2002, pp. 94-96
- Exhibit 6: Boue-Grabot et al., "In Vitro Assesment of Phototoxic Potential Using Human Reconstructed Epidermis" *BIO-HC REPORT*, August 26, 2003, pp. 1-9
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